PMRA Submission Number {......}

EPA MRID No. 48718007

Data Requirement: PMRA Data Code *{.....*}

EPA DP Barcode 402518 OECD Data Point {.....} **EPA MRID** 48718007 **EPA Guideline** 850.1300

Test material: BAS 183 WB H **Purity: 48.41% ai**

Common name: Dicamba BAPMA Salt

Chemical name: IUPAC:

CAS name: N,N-bis(3-aminopropyl)methylamine

105-83-9 (ai) CAS No.:

BAS 183 H LVF, BAAS 183 22 H, BAPMA Dicamba Salt Synonyms:

Signature: Date: 11/21/12
Signature: Mia Howard Primary Reviewer: David A. McEwen

Staff Scientist, CSS-Dynamac Corporation

Secondary Reviewer: Mia Howard

Environmental Scientist, CDM Smith Date: 01/24/13

Primary Reviewer: Elizabeth Donovan, Biologist

EPA/EFED/ERB 6

Date: 9/7/2016

Digitally signed by Elizabeth Donovan

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Reference/Submission No. {.....}

Company Code Active Code [For PMRA] {.....} **Use Site Category** [For PMRA] {.....}

EPA PC Code 100094

Date Evaluation Completed: 11-3-2016

CITATION: Nierzedska, E. 2011. BAS 183 WB H – Daphnia magna Reproduction Test. Unpublished study performed by Department of Ecotoxicology, Institute of Industrial Organic Chemistry, Pszczyna, Poland. Laboratory Project ID: W/05/11. Study sponsored by BASF SE, Crop Protection Division, Agricultural Center Limburgerhof, Germany. Study initiated April 30, 2011 and completed December 21, 2011.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the chronic toxicity of a pesticide to freshwater invertebrates. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

The 21-day-chronic toxicity of BAS 183 WB H (a soluble liquid formulation containing 48.41% dicamba BAPMA salt) to *Daphnia magna* was studied under static-renewal conditions. Daphnids were exposed to BAS 183 WB H at nominal concentrations of 0 (negative control), 18.6, 24.2, 31.4, 40.8, 53.1, 69, and 90 mg/L. Time-weighted mean-measured concentrations were <0.005 (<LOD, control), 8.6, 12, 15, 20, 25, 33, and 43 mg ai/L, equivalent to <LOD (control), 18, 24, 31, 40, 52, 69, and 89 mg formulation/L. No treatment-related effects were observed on adult survival, reproduction (age of first brood release, number of offspring per surviving adult, and intrinsic rate of population increase), or growth (length). The overall NOAEC and LOAEC values were 89 and >89 mg/L (43 and >43 mg ai/L, respectively), respectively, based on time-weighted mean-measured concentrations.

This study is classified as scientifically sound and satisfies guideline requirements for a chronic toxicity study with freshwater invertebrates.

Results Synopsis

Test Organism Age (eg. 1st instar): neonates, <24 hours old

Test Type (Flow-through, Static, Static-Renewal): static-renewal (unaerated)

Time-weighted mean-measured/total formulation:

21-day EC₅₀ (immobility/survival): >89 mg/L 95% C.I.: N/D

NOAEC: 89 mg/L LOAEC: >89 mg/L

Time-weighted mean-measured/ai (dicamba salt):

21-day EC₅₀ (immobility/survival): >43 mg ai/L 95% C.I.: N/D

NOAEC: 43 mg ai/L LOAEC: >43 mg ai/L

Time-weighted mean-measured/ ae (dicamba acid)

21-day EC₅₀ (immobility/survival): >42 mg ae/L 95% C.I.: N/D

NOAEC: 42 mg ae/L LOAEC: >42 mg aieL

Endpoint(s) affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in the OECD

Guidelines for Testing of Chemicals, No. 211 (1998).

There were no notable deviations from U.S. EPA OPPTS 850.1300 guidance.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality

claims statements were provided. This study was conducted in accordance with GLP Standards as published by the U.S. EPA in 40 CFR Part 160

excluding conduct of the preliminary studies.

A. MATERIALS:

1. Test Material BAS 183 WB H [soluble liquid (SL) formulation]

Description: Pale brown liquid

Lot No./Batch No.: 1732-10

Purity: 48.41% ai

Stability of compound

under test conditions: The stability of BAS 183 WB H in dilution water was demonstrated by

regular analysis of fresh and aged solutions throughout the study. Measured

concentrations ranged from 80.7 to 102.6% of nominal levels.

Storage conditions

of Test chemicals: Room temperature in the dark

Physicochemical properties of dicamba.

| Parameter | Values | Comments |
|------------------|---------------|--------------------|
| Water solubility | >250 g/L | At pH 6.8 |
| Vapor pressure | 1.67 mPa | At 25°C |
| UV absorption | Not reported | |
| pKa | Not reported | |
| Kow | logKow = -1.8 | At pH 6.8 and 25°C |

2. Test Organism:

Species: Daphnia magna STRAUS, <24 hours old

EPA and OECD recommend Daphnia magna

Age of the parental stock: Not reported

EPA recommends that young daphnids \leq 24 hours old from a separate parental

culture be used

Source: Laboratory cultures

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EPA requires all test organisms must be produced from laboratory reared culture that has been maintained for at least 21 days at test conditions in dilution water with renewal of the culture medium at least three times per week

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The test concentrations were selected based on the results of four preliminary tests. In the first acute immobilization test, *Daphnia magna* (20/concentration) were exposed to BAS 183 WB H at nominal concentrations of 10 to 100 mg/L. After 48 hours exposure, 60% of the animals at 100 mg/L were immobilized. In the second acute immobilization test, *Daphnia magna* (20/concentration) were exposed to nominal concentrations of 18 to 320 mg/L. After 24 hours exposure, 25 to 80% of the animals at 180 and 320 mg/L were immobilized. After 48 hours exposure, 45 to 100% of the animals at 100 to 320 mg/L were immobilized. In the first 21-day reproduction study, *Daphnia magna* (10/concentration) were exposed to nominal concentrations of 2.4 to 56 mg/L. No toxic effects on the number of offspring produced per surviving adult or mean adult length were observed at any concentration. The high-dose group (56 mg/L) averaged 60.8 offspring/surviving adult compared to 66.7 offspring/surviving adult in the controls. In the second reproduction study, *Daphnia magna* (10/concentration) were exposed to nominal concentrations of 0 and 100 mg/L. At 100 mg/L, 60% of the adult animals were immobilized. No toxic effects on the number of offspring produced per surviving adult or mean adult length were observed at either concentration. The 100 mg/L group averaged 51.7 offspring/surviving adult compared to 54.3 offspring/surviving adult in the controls.

b. Definitive Study

Table 1: Experimental Parameters

| P | 75.4.2 | Remarks | |
|-----------------------------------|---|--|--|
| Parameter | Details | Criteria | |
| Parental acclimation: Period: | Not reported | The age of the parental daphnids was not reported; however, it was stated that the animals used in the study were | |
| Conditions (same as test or not): | Generally same as test | not first brood progeny. | |
| Feeding: | Fed daily with the green algae (Pseudokirchneriella subcapitata) and occasionally supplemented with Spirulina maxima to provide vitamins of group B and micronutrients. | EPA recommends that prior to testing, daphnids that are at least 10-12 days old (those that have had at least one brood) should be separated from the culture, put in separate container and maintained for at least 21 days to insure that good health conditions are | |
| Health (any mortality observed): | No ephippia were present | present | |

| Donomoton | Dataila | Remarks | |
|---|---|---|--|
| Parameter | Details | Criteria | |
| Test condition: | | | |
| Static renewal/flow-through: | Static renewal | | |
| Type of dilution system- for flow through method: | N/A | (EPA requires consistent flow rate of 5- 10 vol/24 hours, meter systems | |
| Renewal rate for static renewal: | Every Monday, Wednesday, and Friday | calibrated before study and checked twice daily during test period) | |
| Aeration, if any: | None | | |
| | | EPA recommends test chambers should not be aerated | |
| Duration of the test: | 21 days | | |
| | | Recommended duration is 21 days. | |
| Test vessel: | | | |
| Material (glass/stainless steel): | Glass | 1. <u>Recommended Material</u> : Glass, No. 316 stainless steel, or | |
| Size (for growth and reproduction/survival test): | 150 mL | perfluorocarbon plastics 2. <u>Recommended Size</u> : 250 ml with 200 ml fill volume; 100 ml with 80 ml | |
| Fill volume: | 90 mL | fill volume OECD guideline recommends that parent animals be maintained individually; one per vessel, with 50 - 100 ml of medium in each vessel. | |
| Source of dilution water: | Elendt M7 medium was | | |
| | prepared by adding stock solutions of reagent-grade chemicals to de-ionized water. The dilution water was aerated prior to use. | Recommended source of dilution water includes unpolluted well or spring water that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details). | |

| D | D 4 2 | Remarks | |
|------------------------------|--|---|--|
| Parameter | Details | Criteria | |
| Water parameters: | | | |
| Hardness: | 170.4 to 219.5 mg/L as CaCO ₃ | | |
| pH: | 7.56 to 7.88 | Recommended hardness: 160 to 180 | |
| Dissolved oxygen: | 8.2 to 9.5 mg/L | mg/L as CaCO3; OECD recommends > | |
| Temperature: | 19.9 to 22.4°C | 140 mg/L as CaCO ₃ Recommended pH: 7.6 to 8.0 pH | |
| Total Organic Carbon: | Not reported | should not deviate by more than 1.0 | |
| Particulate matter: | Not reported | unit for more than 48 hours. OECD | |
| Metals: | Not reported | recommends that pH range be 6 - 9 and | |
| Pesticides: | Not reported | does not vary more than 1.5 units in | |
| Chlorine: | Not reported | any one test. | |
| Chlorine. | Not reported | Recommended dissolved oxygen: renewal should not drop below 50% for | |
| Frequency of measurements: | Dissolved oxygen and pH were measured at all levels three times per week at each solution renewal (both old and new solutions). Temperature was continuously monitored in a surrogate vessel. Hardness was measured once per week in both fresh and spent solutions at all concentrations. | more than 48 hours. Recommended flow-through: \exists 60% throughout test. Recommended temperature: 20°C ± 2°C.; should not deviate from 20°C by more than 5°C for more than 48 hours. OECD recommends a range of 18 - 22°C; temperature should not vary more than ± 2°C OECD guideline recommends that total organic carbon < 2 mg/L | |
| Number of replicates: | | | |
| For growth and reproduction: | 10 replicates per level | NT 1 C 1 1 1 1 1 1 | |
| For survival test: | (Same) | Number of replicates should include a control(s) and at least 5 test concentrations; dilution factor should not be greater than 50%. OECD recommends that at least 5 test concentrations be used in a geometric series with a separation factor not exceeding 3.2. | |
| Number of organisms: | | | |
| For growth and reproduction: | 1 daphnia per replicate | | |
| 3 | | Recommended number of organisms | |
| For survival test: | (Same) | include 22 daphnids/test concentration; 7 test chambers should contain 1 daphnid each, and 3 test chambers contain 5 daphnids each. OECD recommends holding a minimum of 10 daphnids individually for static tests. For flow-through tests, 40 animals should be divided into 4 groups of 10 | |

| Davameter | Dotaile | Remarks |
|--|--|--|
| Parameter | Details | Criteria |
| Treatment Concentrations: Nominal (formulation): | 0 (negative control), 18.6, 24.2, 31.4, 40.8, 53.1, 69.0, and 90.0 mg/L | Although analytical variation was minimal (<±20%) for all levels, the results were expressed in terms of time-weighted mean concentrations as per the Sponsor's request. |
| Nominal (ai): | 0 (negative control), 9.00, 11.72, 15.20, 19.75, 25.71, 33.40, and 43.40 mg ai/L | From the measured dicamba (ai) concentrations, time-weighted mean measured concentrations in terms of |
| Time-weighted mean measured (ai): | <0.005 (<lod, 8.6,<br="" control),="">11.7, 15.0, 19.6, 25.4, 33.2, and 43.2 mg ai/L</lod,> | total formulation were also calculated. |
| Time-weighted mean measured (formulation): | <lod (control),="" 17.69,="" 24.16,="" 30.92,="" 40.47,="" 52.24,="" 68.61,="" 89.32="" and="" l<="" mg="" td=""><td></td></lod> | |
| Solvent (type, percentage, if used) | NA | |
| | | Solvent concentration should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone and ethanol. OECD recommends #0.1 ml/L of solvent. |
| Lighting: | 16 hours light/8 hours dark | Light intensity was measured two or three times per week during the test. |
| | Averaged intensity of 693 lux | Recommended photoperiod is 16 hours light and 8 hours of dark. |
| Recovery of the chemical: | Fresh: 93.6 to 102.5% Aged: 80.7 to 102.6% | Water samples were analyzed for BAS 183 WB H using HPLC with UV-VIS detection. |
| Frequency of determination: | Days 0, 2, 5, 7, 14, and 21 | The LOD was erroneously reported to |
| Limit of Quantification: Limit of Detection: | 0.01 mg ai/L 0.005 mg ai/L | be 0.005 g/L on p. 22 of the study report. It was presumed by the reviewer that the correct units were mg/L. |
| Positive control {if used, indicate the chemical and concentrations} : | Not reported | |

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| Parameter | Details | Remarks | |
|-----------------------------------|---|----------|--|
| | Details | Criteria | |
| Other parameters, if any Feeding: | At the start of the test and each subsequent renewal event, fresh solutions were supplied with algae suspension in the amount sufficient to provide 0.2 mg TOC/daphnid/day. | | |

2. Observations:

Table 2: Observations

| Parameters | Detelle | Remarks | |
|--------------------------------|--|--|--|
| | Details | Criteria | |
| Data endpoints measured (list) | -Parental survival/immobility -First day of reproduction -Number of young produced per surviving female -Intrinsic rate of population growth -Length of surviving adults | Dry weight was not recorded Recommended endpoints measured: - Survival of first-generation daphnids, - Number of young produced per female, - Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test, - Observations of other effects or clinical signs. | |
| Observation intervals | Daily | | |
| Were raw data included? | Yes | | |
| Other observations, if any | None reported | | |

II. RESULTS AND DISCUSSION

A. MORTALITY AND SUBLETHAL EFFECTS:

Adult survival was 100% in the controls and all test concentration during the study. The 21-day EC_{50} for immobilization was >89 mg/L, and the NOAEC and LOAEC values were nominally 89 and >89 mg/L, respectively, based on time-weighted mean measured concentrations (total formulation).

Mean lengths of surviving daphnia ranged from 2.78 to 2.90 mm in all groups, including the control. The 21-day EC₅₀ for growth (i.e., length) was >89 mg/L, and the NOAEC and LOAEC values were nominally 89 and >89 mg/L, respectively, based on time-weighted mean measured concentrations (total formulation).

B. EFFECT ON REPRODUCTION:

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For all levels (including the control), the time to first brood release averaged 8.8 to 9.6 days and the mean number of offspring per surviving adult ranged from 61.1 to 83.4, with no statistically-significant differences indicated at any level for either parameter.

The intrinsic rate of population increase (r) was 0.317/day for the control level, and ranged from 0.326 to 0.353/day for all treatment levels, with no statistically-significant differences from the control indicated.

For all (reproductive) endpoints, the EC₅₀ was >89 mg/L, and the NOAEC and LOAEC values were 89 and >89 mg/L, respectively, using time-weighted mean concentrations (total formulation).

Table 3: Effect of BAS 183 WB H on Growth, Reproduction, and Survival of Daphnia magna. (a)

| Treatment TWA (and Nominal) Concentrations (mg/L) | Adult Survival (%) | Age of First Brood Release (Day ± SD) | Total Live Young/ Surviving Female (No. ± SD) | Adult Length (mm ± SD) ^(b) |
|---|-----------------------|---|---|--|
| Negative Control | 100 | 9.6 ± 0.74 | 61.1 ± 7.95 | 2.78 ± 0.11 |
| 18 (18.6) | 100 | 9.2 ± 0.95 | 83.4 ± 12.97 | 2.88 ± 0.08 |
| 24 (24.2) | 100 | 9.6 ± 1.10 | 66.6 ± 11.40 | 2.78 ± 0.14 |
| 31 (31.4) | 100 | 9.2 ± 0.48 | 69.9 ± 10.59 | 2.81 ± 0.13 |
| 40 (40.8) | 100 | 9.1 ± 0.97 | 82.2 ± 14.85 | 2.87 ± 0.10 |
| 52 (53.1) | 100 | 9.2 ± 0.48 | 73.7 ± 9.37 | 2.87 ± 0.08 |
| 69 (69.0) | 100 | 9.3 ± 0.92 | 79.5 ± 17.70 | 2.90 ± 0.09 |
| 89 (90.0) | 100 | 8.8 ± 0.48 | 77.2 ± 13.39 | 2.81 ± 0.10 |
| NOAEC (mg/L) | 89 | 89 | 89 | 89 |
| LOAEC (mg/L) | >89 | >89 | >89 | >89 |

⁽a) Data were obtained from Tables 13 and 22 on pages 40 and 49 of the study report.

C. REPORTED STATISTICS:

<u>Statistical Method</u>: Endpoints that were statistically analyzed were immobilization of parent animals, age at first brood release, cumulative offspring per surviving adult, intrinsic rate of population increase (r), and adult length. All statistical analyses were performed using ToxRat Professional 2.10 and reported in terms of time-weighted mean-measured formulation concentrations.

Data from each endpoint were assessed for normal distribution using the Shapiro-Wilk's Test and for homogeneity of variance using Levene's Test, and were then subjected to Williams Multiple Sequential t-test Procedure ($\alpha=0.05$). NOAEC and LOAEC values were assigned based on significance.

The EC_x for each response was determined through the use of probit analysis using linear maximum likelihood

⁽b) Data were obtained from statistical output table on page 67 of the study report.

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regression.

Survival/immobility

 EC_{50} (21 d): >89 mg/L 95% C.I.: N/D

NOAEC: 89 mg/L LOAEC: >89 mg/L

Age at first observed reproduction

NOAEC: 89 mg/L LOAEC: >89 mg/L

Number of young/surviving adult

EC₅₀ (21 d): >89 mg/L 95% C.I.: N/D

NOAEC: 89 mg/L LOAEC: >89 mg/L

Intrinsic rate of population growth

 EC_{50} (21 d): >89 mg/L 95% C.I.: N/D

NOAEC: 89 mg/L LOAEC: >89 mg/L

Length

 EC_{50} (21 d): >89 mg/L 95% C.I.: N/D

NOAEC: 89 mg/L LOAEC: >89 mg/L

Endpoint(s) affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The reviewer assessed the endpoints for survival/immobility, age at first observed reproduction, intrinsic rate of growth, number of young/surviving adult, and adult length. Due to the overall lack of immobility in both the control and treatment groups, the EC50, NOAEC, and LOAEC values based on survival/immobility were empirically determined. The intrinsic rate of growth data were visually assessed based on the mean values for each test level, as no raw data were provided. The reviewer statistically analyzed the day of first observed reproduction (see Reviewer's Comments for calculation of the raw data), number of young/surviving adult, and adult length data using Toxstat 3.5 statistical software. These data were assessed for normality and homogeneity of variance using Chi-square and Levene's tests, respectively. The age at first observed reproduction data met the assumption of homogeneity of variance, but not normality. Log base 10 transformation of the data corrected the issue of normality and therefore transformed age at first observed reproduction data were analyzed using ANOVA followed by Dunnett's test. The number of young/surviving adult and adult length data met all assumptions of normality and homogeneity without transformation and were therefore analyzed, untransformed, using ANOVA followed by Dunnett's tests. The toxicity values are reported in terms of time-weighted mean formulation concentrations.

Survival/immobility

EC₅₀ (21 d): >89 mg/L 95% C.I.: N/D

NOAEC: 89 mg/L LOAEC: >89 mg/L

Age at first observed reproduction

NOAEC: 89 mg/L

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LOAEC: >89 mg/L

Number of young/surviving adult

NOAEC: 89 mg/L LOAEC: >89 mg/L

Intrinsic rate of population growth

NOAEC: 89 mg/L LOAEC: >89 mg/L

Length

NOAEC: 89 mg/L LOAEC: >89 mg/L

E. STUDY DEFICIENCIES:

There were no significant deviations or deficiencies from OCSPP 850.1300 guidance.

F. REVIEWER'S COMMENTS:

The reviewer's results agreed with those of the study author. In the Executive Summary and Conclusions sections of the DER, results were provided in terms of time-weighted mean measured concentrations, as mg ai (dicamba)/L and mg (formulation)/L. Results were rounded to two significant figures.

All validity requirements were met. Specifically, $1 \le 20\%$ of the control organisms appeared to be immobilized, stressed, or diseased during the test; 2) each surviving control daphnid produced an average of ≥ 60 young; and 3) no ephippia were produced by control animals.

The reviewer calculated the raw age of first reproduction data using the values in Table 14 through 21 of the study report. The reviewer extracted the day of first reproduction raw data from these tables and then added 0.5 day to each individual value. It appears that the study author assumed that each daphnid was 0.5 day old at test initiation (day 0) of the test, based on the means reported on page 68 of the study report . By adding 0.5 to each day of first reproduction, the reviewer was able to calculate mean ($\pm SD$) age of first reproduction values that matched those reported by the study author.

The intrinsic rate of population growth (r) integrates results of reproductive output and age-specific mortality; the units for (r) are 1/day. The formula for calculation of (r) was not reported and replicate data were not reported. Therefore, the reviewer analyzed the endpoints for intrinsic rate of population growth visually, based on the mean values provided by the study author on page 70 of the study report. Because stimulation of the mean growth rate was observed at all test levels relative to the negative control, the reviewer determined that there were no adverse effects.

The in-life study dates were May 4 to May 25, 2011.

G. CONCLUSIONS:

This study is scientifically sound and is thus acceptable. No treatment-related effects were observed on adult survival, reproductive parameters, or growth (length).

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<u>Time-weighted mean-measured/total formulation:</u>

21-day EC₅₀ (immobility/survival): >89 mg/L 95% C.I.: N/D

NOAEC: 89 mg/L LOAEC: >89 mg/L

Time-weighted mean-measured/ai (dicamba):

21-day EC₅₀ (immobility/survival): >43 mg ai/L 95% C.I.: N/D

NOAEC: 43 mg ai/L LOAEC: >43 mg ai/L

Time-weighted mean-measured/ ae (dicamba acid)

21-day EC₅₀ (immobility/survival): >42 mg ai/L 95% C.I.: N/D

NOAEC: 42 mg ai/L LOAEC: >42 mg ai/L

Endpoint(s) affected: None

III. REFERENCES:

OECD. 2008 (October 3). OECD Guideline for Testing of Chemicals No. 211 "Daphnia magna Reproduction Test".

Directive 2001/59/EC, Appendix No V, Part C: C.20." Daphnia magna reproduction test".

OECD. 1997. OECD Environmental Health and Safety Publications, Series on Testing and Assessment, No. 6, "Report of the Final Ring Test of the Daphnia magna Reproduction Test", Environment Directorate. Paris.

ToxRat Professional ver. 2.10.

Directive 2004/10/EC on the harmonization of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances (codified version).

Polish legislation: regulation of the Minister of Health of June 03, 2003, Dz. U. No.116, Poz.1103, regulation of the Minister of Health of 28th May 2010 concerning the criteria that must be met by institutions conducting tests of chemical substances and preparations, and the verification of compliance with these criteria (Dz.U. Nr 109, poz. 722).

PN-EN ISO 6059: 1999 Water quality – Determination of the sum of calcium and magnesium – EDTA titrimetric method

OECD Guideline for Testing of Chemicals No 211 (1998): 'Daphnia sp. reproduction test' Annex No. 6. The calculations of the time –weighted mean.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION

| Title: | Dicamba chronic | daphnid age | at first reproduction | |
|--------|-----------------|-------------|-----------------------|----------------|
| File: | 8007b | | Transform: | LOG BASE 10(Y) |

Chi-Square Test for Normality

Actual and Expected Frequencies

| INTERVAL | <-1.5 | -1.5 to <-0.5 | -0.5 to 0.5 | >0.5 to 1.5 | >1.5 |
|----------|-------------------|-----------------|---------------|-------------|--------|
| | and man area area | | | | |
| EXPECTED | 5.3600 | 19.3600 | 30.5600 | 19.3600 | 5.3600 |
| OBSERVED | 1 | 31 | 25 | 17 | 6 |

Critical Chi-Square =
$$13.277$$
 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba chronic daphnid age at first reproduction Transform: LOG BASE 10(Y) File: 8007b

Levene's Test for Homogeneity of Variance

ANOVA Table

| SOURCE | DF | SS | MS | F |
|----------------|----|--------|--------|--------|
| Between | 7 | 0.0050 | 0.0007 | 0.7993 |
| Within (Error) | 72 | 0.0647 | 0.0009 | |
| Total | 79 | 0.0697 | | |

(p-value = 0.5905)

Critical F =
$$2.8983$$
 (alpha = 0.01 , df = $7,72$)
= 2.1397 (alpha = 0.05 , df = $7,72$)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba chronic daphnid age at first reproduction

File: 8007b Transform: NO TRANSFORMATION

ANOVA Table

| A Submission Number {} | | | | |
|------------------------|----|--|----------|-----------|
| SOURCE | DF | SS | MS | F |
| Between | 7 | 4.8000 | 0.6857 | 1.0686 |
| Within (Error) | 72 | 46.2000 | 0.6417 | |
| Total | 79 | 51.0000 | | |
| | | | (p-value | = 0.3924) |
| Critical F = 2 = 2 | | pha = 0.01, df = 7,72) pha = 0.05, df = 7,72) | | |

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Dicamba chronic daphnid age at first reproduction File: 8007b Transform: NO TRANSFORMATION

| | Dunnett's Test - | TABLE 1 OF 2 | Ho:Control> | [reatment | |
|-------|------------------|---------------------|--------------------------------------|-----------------|-----|
| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | TRANS T STAT | SIG |
| 1 | neg control | 9.6000 | 9,6000 | | |
| 2 | 18 | 9.2000 | 9.2000 | -1.1166 | |
| 3 | 24 | 9.6000 | 9.6000 | 0.0000 | |
| 4 | 31 | 9.2000 | 9.2000 | -1.1166 | |
| 5 | 40 | 9.1000 | 9.1000 | -1.3957 | |
| 6 | 52 | 9.2000 | 9.2000 | -1.1166 | |
| 7 | 69 | 9.3000 | 9.3000 | -0.8374 | |
| 8 | 89 | 8.8000 | 8.8000 | -2.2332 | |
| | | | | | |

Dunnett critical value = 2.4000 (1 Tailed, alpha = 0.05, df [used] = 7,60) (Actual df = 7,72)

Title: Dicamba chronic daphnid age at first reproduction File: 8007b Transform: NO TRANSFORMATION

| | Dunnett's Test - | TABLE 2 | OF 2 Ho | :Control> | Treatment |
|-------|------------------|----------------|---|---------------------------------------|---|
| GROUP | IDENTIFICATION | NUM OF REPS | MIN SIG DIFF (IN ORIG. UNITS) | % OF CONTROL | DIFFERENCE FROM CONTROL |
| 1 | neg control | 10 | the tab tab tab and the tab tab tab tab tab tab and tab tab tab tab tab | AND DESCRIPTION OF THE PARTY NAME AND | 300 500 500 500 500 500 500 500 500 500 |
| 2 | 18 | 10 | 999.9999 | 0.0 | -0.4000 |
| 3 | 24 | 10 | 999.9999 | 0.0 | -0.0000 |
| 4 | 31 | 10 | 999.9999 | 0.0 | -0.4000 |
| 5 | 40 | 10 | 999.9999 | 0.0 | -0.5000 |
| 6 | 52 | 10 | 999.9999 | 0.0 | -0.4000 |
| 7 | 69 | 10 | 999.9999 | 0.0 | -0.3000 |
| 8 | 89 | 10 | 999.9999 | 0.0 | -0.8000 |

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NOTE: MSD = 999.9999 means actual MSD estimate > 999.

Title: Dicamba chronic daphnid offspring/surviving adult

File: 8007r Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

OBSERVED 5 21 27 22

Chi-Square = 0.9620(p-value = 0.9155)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4) = 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba chronic daphnid offspring/surviving adult

Transform: File: 8007r NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

DF SOURCE SS MS ______ 697.5500 99.6500 1.5553 Between

Within (Error) 72 4613.0000 64.0694 ______

Total 79 5310.5500

(p-value = 0.1628)

Critical F = 2.8983 (alpha = 0.01, df = 7,72) = 2.1397 (alpha = 0.05, df = 7,72)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

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| Title: | Dicamba | chronic | daphnid | offspring/ | 'surviving | adult |
|--------|---------|---------|---------|------------|------------|-------|
| TO 2 7 | | 0007 | | m 4 | | |

File: 8007r Transform: NO TRANSFORMATION

ANOVA Table

| Within (Error) 72 11472.4000 159.3389 | SOURCE | DF | SS | MS | F |
|---------------------------------------|----------------|----|------------|----------|--------|
| | Between | 7 | 4338.4000 | 619.7714 | 3.8896 |
| | Within (Error) | 72 | 11472.4000 | 159.3389 | |
| Total 79 15810.8000 | Total | 79 | 15810.8000 | | |

(p-value = 0.0012)

Critical F = 2.8983 (alpha = 0.01, df = 7,72) = 2.1397 (alpha = 0.05, df = 7,72)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Dicamba chronic daphnid offspring/surviving adult

File: 8007r Transform: NO TRANSFORMATION

| | Dunnett's Test - | TABLE 1 OF 2 | Ho:Control< | Ireatment | |
|---------------------------------|---|---|---|--|-----|
| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
| 1 2 3 4 5 6 7 | neg control 18 24 31 40 52 69 | 61.1000 83.4000 66.6000 69.9000 82.2000 73.7000 79.5000 | 61.1000 83.4000 66.6000 69.9000 82.2000 73.7000 79.5000 | -3.9503 -0.9743 -1.5589 -3.7377 -2.2320 -3.2594 | |
| 8 | 89 | 77.2000 | 77.2000 | -2.8520 | |

Dunnett critical value = 2.4000 (1 Tailed, alpha = 0.05, df [used] = 7,60) (Actual df = 7,72)

Title: Dicamba chronic daphnid offspring/surviving adult

File: 8007r Transform: NO TRANSFORMATION

| | Dunnett's Test - | TABLE 2 C |)F 2 Ho | c:Control< | Treatment |
|-------|------------------|----------------|-------------------------------|-----------------|----------------------------|
| GROUP | IDENTIFICATION | NUM OF REPS | MIN SIG DIFF (IN ORIG. UNITS) | % OF CONTROL | DIFFERENCE FROM CONTROL |
| 1 | neg control | 10 | 10 5404 | | |
| 2 | 18 | ΤΟ | 13.5484 | 22.2 | -22.3000 |

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| PMRA Submission Number {} | | | | EPA MRID No. 48718007 | | |
|---------------------------|----|----|---------|-----------------------|----------|--|
| 3 | 24 | 10 | 13.5484 | 22.2 | -5.5000 | |
| 4 | 31 | 10 | 13.5484 | 22.2 | -8.8000 | |
| 5 | 40 | 10 | 13.5484 | 22.2 | -21.1000 | |
| 6 | 52 | 10 | 13.5484 | 22.2 | -12.6000 | |
| 7 | 69 | 10 | 13.5484 | 22.2 | -18.4000 | |
| 8 | 89 | 10 | 13.5484 | 22.2 | -16.1000 | |

Title: Dicamba chronic daphnid offspring/surviving adult

File: 8007r Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

| INTERVAL | <-1.5 | -1.5 to <-0.5 | -0.5 to 0.5 | >0.5 to 1.5 | >1.5 |
|----------|--------|-----------------|-------------|-------------|--------|
| | | | | | |
| EXPECTED | 5.3600 | 19.3600 | 30.5600 | 19.3600 | 5.3600 |
| OBSERVED | 5 | 21 | 27 | 22 | 5 |

Chi-Square = 0.9620 (p-value = 0.9155)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4) = 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba chronic daphnid offspring/surviving adult

File: 8007r Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

 SOURCE
 DF
 SS
 MS
 F

 Between
 7
 697.5500
 99.6500
 1.5553

 Within (Error)
 72
 4613.0000
 64.0694

Total 79 5310.5500

(p-value = 0.1628)

Critical F = 2.8983 (alpha = 0.01, df = 7,72) = 2.1397 (alpha = 0.05, df = 7,72)

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PMRA Submission Number {......}

EPA MRID No. 48718007

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba chronic daphnid age at first reproduction

File: 8007b Transform: NO TRANSFORMATION

ANOVA Table

| SOURCE | DF | SS | MS | F |
|----------------|----|---------|--------|--------|
| Between | 7 | 4.8000 | 0.6857 | 1.0686 |
| Within (Error) | 72 | 46.2000 | 0.6417 | |
| Total | 79 | 51.0000 | | |

(p-value = 0.3924)

Critical F = 2.8983 (alpha = 0.01, df = 7,72) = 2.1397 (alpha = 0.05, df = 7,72)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Dicamba chronic daphnid age at first reproduction

8007b Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control>Treatment ______ TRANSFORMED MEAN CALCULATED IN TRANS SIG MEAN ORIGINAL UNITS T STAT GROUP IDENTIFICATION 0.05 _____ neg control 9.6000 18 9.2000 24 9.6000 31 9.2000 40 9.1000 52 9.2000 69 9.3000 89 8.8000 9.6000 9.2000 9.6000 -1.1166 3 0.0000 4 9.2000 -1.1166 5 9.1000 -1.3957 -1.1166 6 9.2000 7 9.3000 -0.8374 8.8000 -2.2332

Dunnett critical value = 2.4000 (1 Tailed, alpha = 0.05, df [used] = 7,60) (Actual df = 7,72)

Title: Dicamba chronic daphnid age at first reproduction File: 8007b Transform:

NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control>Treatment

NUM OF MIN SIG DIFF % OF DIFFERENCE

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PMRA Submission Number {......}

EPA MRID No. 48718007

| GROUP | IDENTIFICATION | REPS | (IN ORIG. UNITS) | CONTROL | FROM CONTROL |
|-----------------------|----------------------------|----------------------|--|--------------------------|---|
| 1 2 3 | neg control 18 24 | 10 10 10 | 999.9999 | 0.0 | -0.4000 |
| 4 5 6 7 8 | 31 40 52 69 89 | 10 10 10 10 | 999.9999 999.9999 999.9999 999.9999 | 0.0 0.0 0.0 0.0 | -0.4000 -0.5000 -0.4000 -0.3000 -0.8000 |

NOTE: MSD = 999.9999 means actual MSD estimate > 999.

Title: Dicamba chronic daphnid adult lenghths

File: 80071 Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

| INTERVAL | <-1.5 | -1.5 to <-0.5 | -0.5 to 0.5 | >0.5 to 1.5 | >1.5 |
|----------|--------|-----------------|---------------|-------------|--------|
| | | | | | |
| EXPECTED | 5.3600 | 19.3600 | 30.5600 | 19.3600 | 5.3600 |
| OBSERVED | 6 | 17 | 33 | 20 | 4 |

Chi-Square = 0.9252 (p-value = 0.9209)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4) = 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba chronic daphnid adult lenghths

File: 80071 Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

| SOURCE | DF | SS | MS | F |
|----------------|----|--------|--------|--------|
| Between | 7 | 0.0206 | 0.0029 | 0.5774 |
| Within (Error) | 72 | 0.3669 | 0.0051 | |
| Total | 79 | 0.3875 | | |

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(p-value = 0.7720)

Critical F = 2.8983 (alpha = 0.01, df = 7,72) = 2.1397 (alpha = 0.05, df = 7,72)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba chronic daphnid adult lenghths

Transform: NO TRANSFORMATION File: 80071

ANOVA Table

| SOURCE | DF | SS | MS | F |
|----------------|----|--------|--------|--------|
| Between | 7 | 0.1422 | 0.0203 | 1.7802 |
| Within (Error) | 72 | 0.8216 | 0.0114 | |
| Total | 79 | 0.9638 | | |

(p-value = 0.1045)

Critical F = 2.8983 (alpha = 0.01, df = 7,72) = 2.1397 (alpha = 0.05, df = 7,72)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Dicamba chronic daphnid adult lenghths

NO TRANSFORMATION 80071 File: Transform:

| | Dunnett's Test - | TABLE 1 OF 2 | Ho:Control <treatment< th=""></treatment<> | | |
|---------------------------------|---|--|--|---|-----|
| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
| 1 2 3 4 5 6 7 | neg control 18 24 31 40 52 69 | 2.7780 2.8600 2.7840 2.8140 2.8680 2.8720 2.9000 2.8100 | 2.7780 2.8600 2.7840 2.8140 2.8680 2.8720 2.9000 2.8100 | -1.7165 -0.1256 -0.7536 -1.8840 -1.9677 -2.5538 -0.6699 | |

Dunnett critical value = 2.4000 (1 Tailed, alpha = 0.05, df [used] = 7,60) (Actual df = 7,72)

Title: Dicamba chronic daphnid adult lenghths

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EPA MRID No. 48718007 PMRA Submission Number {......} Transform: File: 80071 NO TRANSFORMATION Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment NUM OF MIN SIG DIFF % OF DIFFERENCE GROUP IDENTIFICATION REPS (IN ORIG. UNITS) CONTROL FROM CONTROL (IN ORIG. UNITS) CONTROL FROM CONTROL neg control 10

18 10 0.1147 4.1 -0.0820
24 10 0.1147 4.1 -0.0360
31 10 0.1147 4.1 -0.0360
40 10 0.1147 4.1 -0.0900
52 10 0.1147 4.1 -0.0940
69 10 0.1147 4.1 -0.1220
89 10 0.1147 4.1 -0.1220 3 4 5 7